

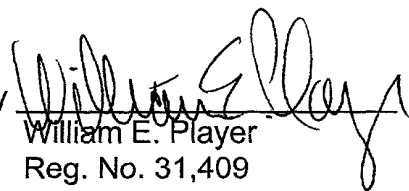
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED / ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER P67341US0
		US APPLICATION NO. (if known, see 37 CFR 1.51) 097926589
INTERNATIONAL APPLICATION NO. PCT/FR00/01382	INTERNATIONAL FILING DATE 19 May 2000	PRIORITY DATE CLAIMED 21 May 1999
TITLE OF INVENTION TETRAMIDES OF A GADOLINIUM COMPLEX AND APPLICATION IN MEDICAL IMAGING		
APPLICANT(S) FOR DO/EO/US Olivier ROUSSEAUX and Christian SIMONOT		

Applicant herein submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for Internatl. Preliminary Examination was made by the 19th month from earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the Internatl. Preliminary Examination report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
 - International Search Report - EPO
 - First Page of Publication
 - Amended set of claims as filed with the International Preliminary Examination Report

US APPLICATION NO. (If known, see 37 CFR 1.5) <div style="font-size: 1.5em; font-weight: bold;">09/926589</div>		INTERNATIONAL APPLICATION NO <div style="font-weight: bold;">PCT/FR00/01382</div>		ATTORNEY'S DOCKET NUMBER <div style="font-weight: bold;">P67341US0</div>	
17. <input checked="" type="checkbox"/> The following fees are submitted: Basic National Fee (37 CFR 1.492(a)(1)-(5)): Internatl. prelim. examination fee paid to USPTO (37 CFR 1.492 (a) (1)) .. \$710.00 No international preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (2)) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .. \$740.00 Neither international preliminary examination fee (37 CFR 1.492 (a) (3)) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO) \$1040.00 International preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (4)) and all claims satisfied provisions of PCT Article 33(2)-(4) \$100.00 Search Report prepared by the EPO or JPO (37 CFR 1.492 (a) (5)) \$890.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>				CALCULATIONS \$ 890.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
Claims	Number Filed	Number Extra	Rate		
Total Claims	8 - 20 =	-0-	x \$18.00	\$	
Independent Claims	2 - 3 =	-0-	x \$84.00	\$	
Multiple Dependent Claim(s) (if applicable)			+ \$280.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$ 890.00	
Reduction by 1/2 for filing by small entity , if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				\$	
SUBTOTAL =				\$ 890.00	
Processing fee of \$130 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f))				\$	
TOTAL NATIONAL FEE =				\$ 890.00	
Fee of \$40.00 for recording the enclosed assignment (37 CFR 1.21(h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31).				\$ 40.00	
TOTAL FEES ENCLOSED =				\$ 930.00	
				Amt. to be refunded:	\$
				Amt. charged:	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>930.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. <u>06-1358</u> in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge my account any additional fees set forth in §1.492 during the pendency of this application, or credit any overpayment to Deposit Account No. <u>06-1358</u> . A duplicate copy of this sheet is enclosed.					
SEND ALL CORRESPONDENCE TO:					
JACOBSON HOLMAN PLLC 400 7th Street, N.W., Suite 600 Washington, DC 20004 202-638-6666 CUSTOMER NUMBER: 00136			By  William E. Player Reg. No. 31,409		

09/926589

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Olivier ROUSSEAUX et al.

Serial No.: New

Filing Date: November 21, 2001

For: TETRAMIDES OF A GADOLINIUM COMPLEX AND APPLICATION
IN MEDICAL IMAGING

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

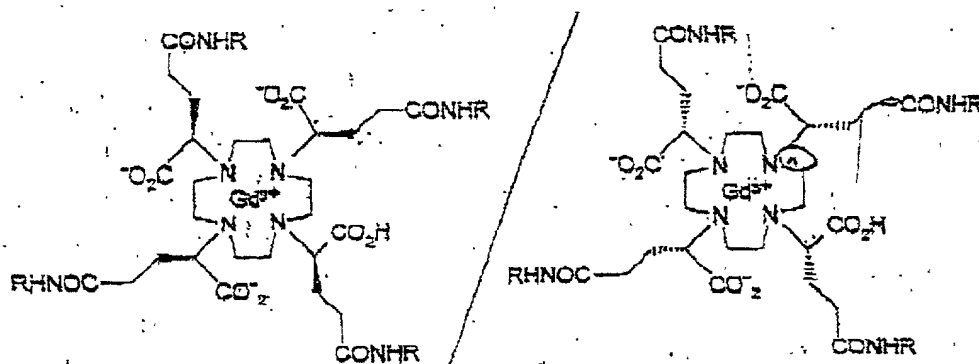
IN THE SPECIFICATION

On page 1, immediately following the title, please insert the following sentence: --This is a 371 of PCT/FR00/01382 filed May 19, 2000, the disclosure of which is incorporated herein by reference---.

IN THE CLAIMS

Please cancel claims 1 through 8 and add new claims 9 through 16 submitted herewith as on attached sheets.

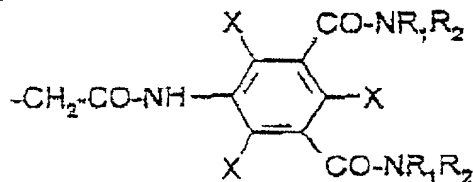
9. Contrast agent for medical imaging, characterized in that it comprises a racemic compound of formula A



in which R is a phenyl group or (C₁-C₈) alkyl group which are substituted or interrupted by one or more groups selected from the group consisting of phenyl, alkyl, oxy, amino and amido groups, which may or may not be substituted by alkyl,

it being possible for the phenyl groups also to be substituted by one or more groups selected from the group selected from OH, Br, Cl, I, (C₁-C₈)alkyl, (C₁-C₈)alkyleneoxy, NO₂, NR_xR_y, NR_xCOR_y, CONR_xR_y and COOR_x, R_x and R_y being (C₁-C₈)alkyl or H, and it being possible for the linear or branched or cyclic alkyl groups to be hydroxylated, and the salts of this acid with a physiologically acceptable inorganic or organic base.

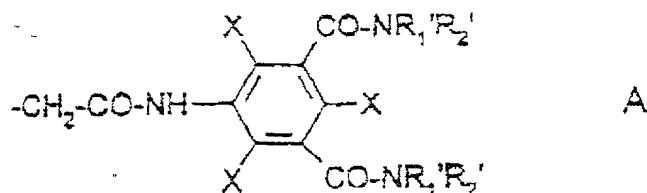
10. Contrast agent according to Claim 9, for which R is a group of formula



in which

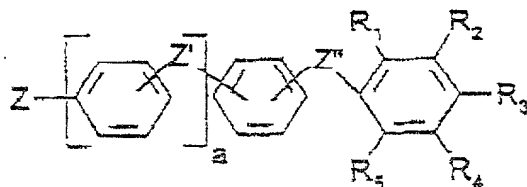
X is Br or I, R₁ is H, (C₁-C₃)alkyl or (C₂-C₈)mono- or polyhydroxyalkyl and R₂ is (C₂-C₈)mono- or

polyhydroxyalkyl, or else R_1 is H and R_2 is a group of formula



X being as defined above and R_1' and R_2' taking any one of the meanings given for R_1 and R_2 , with the exception of A, it being understood that $\text{---CO-NR}_1\text{R}_2$ or $\text{---CO-NR}_1'\text{R}_2'$ comprise at least two hydroxyl groups, and its salts with a physiologically acceptable inorganic or organic base.

11. Contrast agent according to Claim 9, for which R is a group of formula



in which a is 1 or 2,

Z is selected from the group consisting of a bond, CH_2 , CH_2CONH and $(\text{CH}_2)_2\text{NHCO}$,

Z' is selected from the group consisting of a bond, O, S, NQ, CH_2 , CO, CO-NQ , NQ-CO , NQ-CO-NQ and $\text{CO-NQ-CH}_2\text{-CONQ}$,

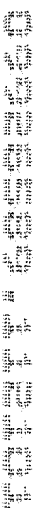
Z'' is selected from the group consisting of CO-NQ , NQ-CO , $\text{CO-NQ-CH}_2\text{-CO-NQ}$ and $\text{NQ-CO-CH}_2\text{-NQ-CO}$,

with Q being H or an optionally hydroxylated $(\text{C}_1\text{--C}_4)$ alkyl group,

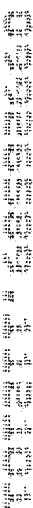
R_1 , R_2 , R_3 , R_4 and R_5 , independently of one another, are selected from the group consisting of H, Br, Cl, I, $\text{CO-NQ}_1\text{Q}_2$ or $\text{N(Q}_1\text{)-CO-Q}_2$, and Q_1 and Q_2 , which are identical or different, are selected from the group consisting of optionally hydroxylated $(\text{C}_2\text{--C}_6)$ alkyl groups optionally interrupted by an oxygen atom, so that Q_1 and Q_2 together comprise

[illegible]

- [illegible]



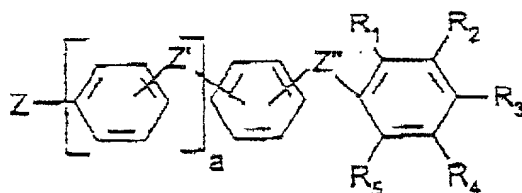
- [illegible]

[illegible]

- [illegible]

its reflux temperature for 35 to 45 hours at pH 3.

15. Racemic compound, for which R is a group of formula



in which a is 1 or 2,

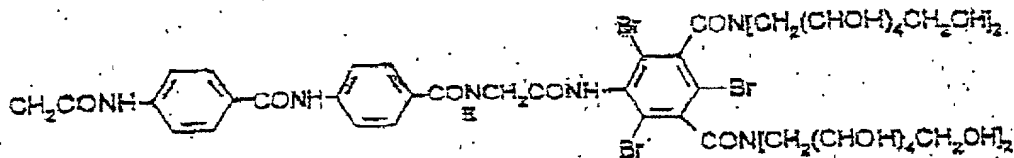
Z is selected from the group consisting of a bond, CH_2 , CH_2CONH and $(\text{CH}_2)_2\text{NHCO}$,

Z' is selected from the group consisting of a bond, O, S, NQ, CH_2 , CO, CO-NQ, NQ-CO, NQ-CO-NQ and $\text{CO-NQ-CH}_2\text{-CONQ}$,

Z'' is selected from the group consisting of a bond CO-NQ, NQ-CO, $\text{CO-NQ-CH}_2\text{-CO-NQ}$ and $\text{NQ-CO-CH}_2\text{-NQ-CO}$, with Q being H or an optionally hydroxylated $(\text{C}_1\text{-C}_4)$ alkyl group,

R_1 , R_2 , R_3 , R_4 and R_5 , independently of one another, are selected from the group consisting of H, Br, Cl, I, $\text{CO-NQ}_1\text{Q}_2$ and $\text{N(Q}_1\text{)-CO-Q}_2$, and Q_1 and Q_2 , which are identical or different, are selected from optionally hydroxylated $(\text{C}_2\text{-C}_6)$ alkyl groups optionally interrupted by an oxygen atom, so that Q_1 and Q_2 together comprise from 4 to 10 OH groups, it being understood that at least 1 and at most 2 R_1 , R_2 , R_3 , R_4 and R_5 groups are amide groups.

16. Racemic compound according to Claim 15, in which R is a group of formula



REMARKS

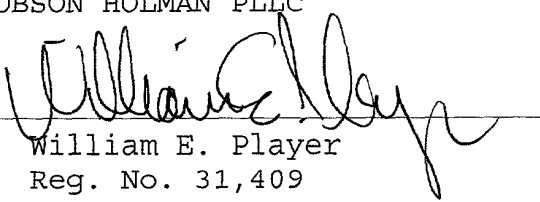
The foregoing Preliminary Amendment is submitted to eliminate multiple dependencies and in order to place the application in better form for examination.

Early action on the merits is respectfully requested.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By


William E. Player
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Atty. Docket: P67341US0
Date: November 21, 2001
WEP/cmf

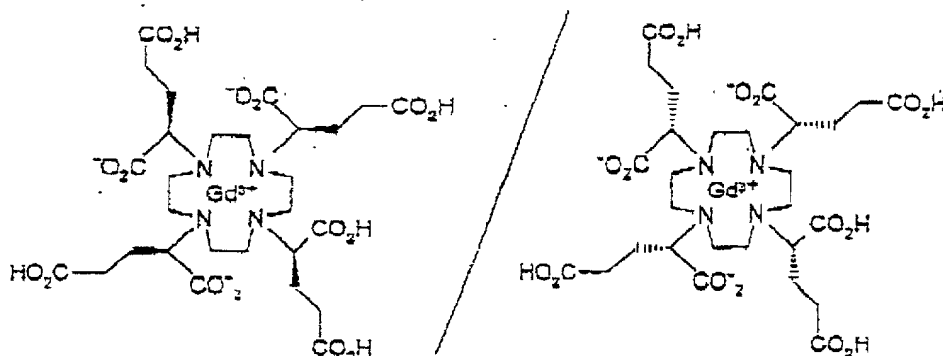
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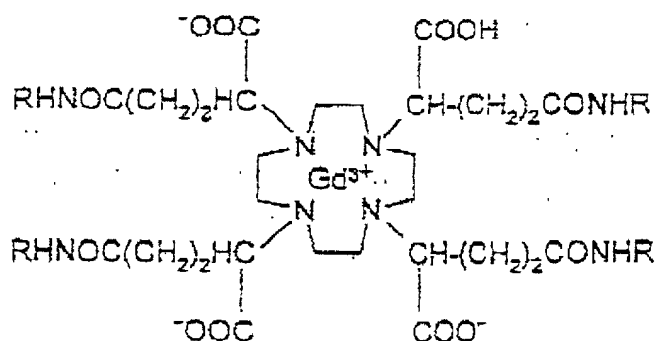
PCT/FR00/01382

TETRAMIDES OF A GADOLINIUM COMPLEX AND APPLICATION IN
MEDICAL IMAGING

The present invention relates to tetramides which are
5 derived from the pair of RRRR/SSSS enantiomers of
tetra(α -carboxyethyl)gadoterate, represented by the
formulae

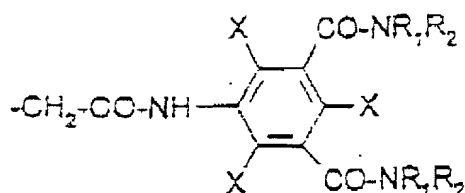


10 It is disclosed in EP 0 661 279 that the amides of
formula II

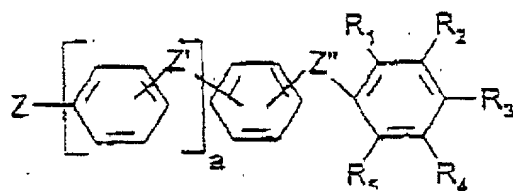


in which R is a bulky hydrophilic group with a
molecular mass of greater than 200, exhibit a
15 longitudinal relaxivity r_1 which is markedly superior
to those of the chelates not carrying the bulky side
group $(CH_2)_2CONHR$ and can be used as contrast agents in
magnetic resonance diagnostic imaging.

20 WO 97/01359 relates to the products of formula II in
which R is a group of formula



Application EP 98 403108 of 9 December 1998 relates to the products of formula II in which R is the following group



It is known that the relaxivity r_1 of a gadolinium chelate is a complex function of various more or less independent factors, including the electronic correlation time, rotation correlation time and water exchange time, which factors depend in particular on the spatial structure of the chelating agent around the paramagnetic cation, so that 2 stereoisomers can have substantially different relaxivities.

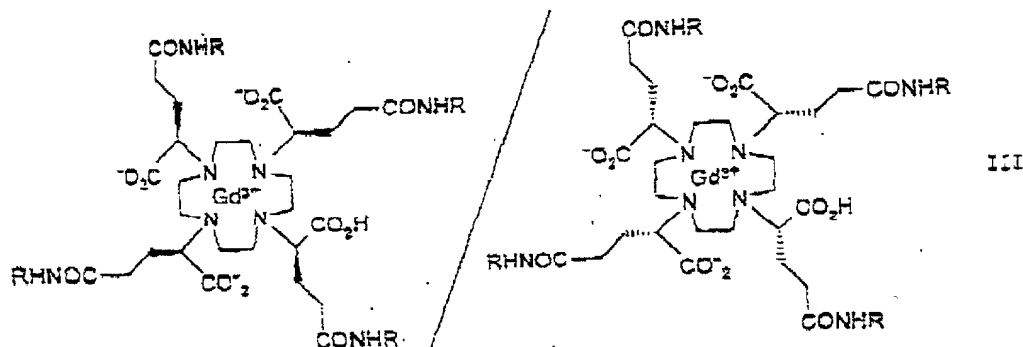
Furthermore, it is essential for the specific characteristics of a pharmaceutical product to be reproducible in terms of effectiveness and of toxicity between successive manufacturing batches and it can be difficult to ensure such reproducibility in the presence of numerous stereoisomers because of their substantial differences in chemical reactivity and in physical properties.

It was thus desirable to find a process which makes it possible, at the industrial stage under acceptable economic conditions, to obtain a mixture of stereoisomers of the amides of formula II in exactly defined proportions and thus to isolate, with good yields, one of the possible racemic compounds which

does not comprise the other stereoisomers and which exhibits an advantageous relaxivity r_1 in the range of the fields currently used clinically, namely between 0.5 and 1.5 tesla.

5

The racemic compounds according to the invention are represented by the formula III



10

in which R is a phenyl group or (C_1-C_8) alkyl group which are substituted or interrupted by one or more groups selected from phenyl, alkyl, oxy, amino or amido groups, which may or may not be substituted by alkyl,

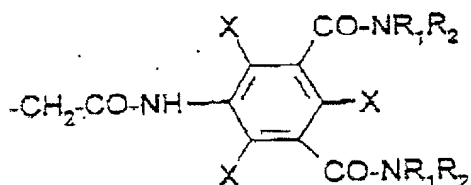
15 it being possible for the phenyl groups to be substituted by OH, Br, Cl, I, (C_1-C_8) alkyl, (C_1-C_8) alkyleneoxy, NO_2 , NR_xR_y , NR_xCOR_y , $CONR_xR_y$ or $COOR_x$, R_x and R_y being (C_1-C_8) alkyl or H,

20 and it being possible for the linear or branched or cyclic alkyl groups to be hydroxylated,

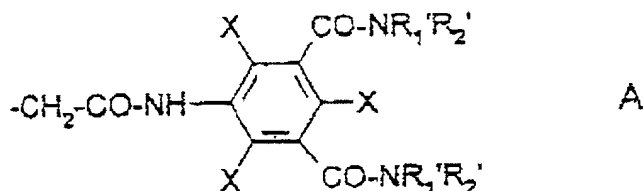
and the salts of these acids with inorganic or organic bases, such as NaOH, KOH, N-methylglucamine, tris-(hydroxymethyl)aminomethane, lysine or diethanolamine.

25 Preference is given, among these, to the compounds in which

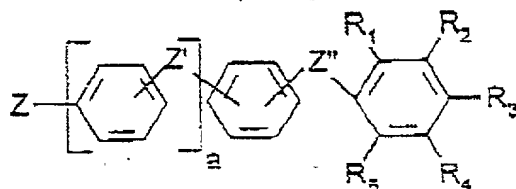
R is a group of formula



in which X is Br or I, R₁ is H, (C₁-C₃)alkyl or (C₂-C₈)mono- or polyhydroxyalkyl and R₂ is (C₂-C₈)mono- or polyhydroxyalkyl, or else R₁ is H and R₂ is a group of formula



X being as defined above and R'₁ and R'₂ taking any one of the meanings given for R₁ and R₂, with the exception of A, it being understood that -CO-NR₁R₂ or -CO-NR'₁R'₂ comprise at least two hydroxyl groups, and those in which R is a group



in which a is 1 or 2,

Z is a bond, CH₂, CH₂CONH or (CH₂)₂NHCO,

Z' is a bond, O, S, NQ, CH₂, CO, CO-NQ, NQ-CO, NQ-CO-NQ or CO-NQ-CH₂-CONQ,

Z'' is CO-NQ, NQ-CO, CO-NQ-CH₂-CO-NQ or NQ-CO-CH₂-NQ-CO, with Q being H or an optionally hydroxylated (C₁-C₄)alkyl group,

R₁, R₂, R₃, R₄ and R₅, independently of one another, are selected from H, Br, Cl, I, CO-NQ₁Q₂ or N(Q₁)-CO-Q₂, and Q₁ and Q₂, which are identical or different, are selected from optionally hydroxylated (C₂-C₆)alkyl groups optionally interrupted by an oxygen atom, so that Q₁ and Q₂ together comprise from 4 to 10 OH groups, it being understood that at least 1 and at most 2 R₁, R₂, R₃, R₄ and R₅ groups are amide groups.

The racemic compounds of the invention can be prepared by a method known per se by reaction of the amine RNH₂ with the pair of complexes of the enantiomeric octaacids of formula I in aqueous solution with an

agent which activates carboxyl functional groups under conventional conditions for peptide condensations, as disclosed in the abovementioned patents, for mixtures of isomers.

5

Some of the isomers of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(2-glutaric acid), obtained by hydrolysis of the corresponding ethyl esters, separated by silica liquid chromatography and crystallization from water, 10 have been described by Judith A.K. Howard et al. in Chem. Commun., 1381-1382 (1998).

A process which can be operated industrially has now been found which makes it possible to obtain the pair 15 of RRRR/SSSS enantiomers starting from the mixture of the stereoisomers of the gadolinium complex of this octaacid resulting from the substitution, by a conventional method, of the nitrogen atoms of 1,4,7,10-tetraazacyclododecane. It consists in carrying out the 20 isomerization by simple heating in aqueous solution at acidic pH, preferably between 2 and 4.5 and better still between 2.5 and 3.5, and at a temperature of greater than 70°C, preferably of greater than 90°C and better still at reflux of the solution, for the time 25 needed to obtain the racemic compound of the invention, i.e. from a few hours to a few days, in particular 35 to 45 hours at reflux at approximately pH 3.

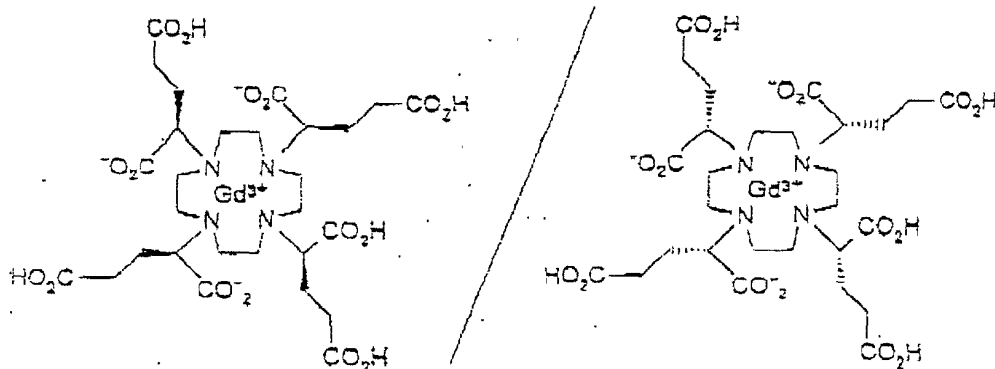
The starting mixture of the stereoisomers can be 30 obtained by the action of the compound of formula $R'OOC-CHX-(CH_2)_2-COOR'$, in which $R' = H$ or (C_1-C_3) alkyl and X is a leaving group in a nucleophilic substitution, in particular a halogen atom, preferably bromine, or a sulphonate or tosylate or triflate group, 35 which reaction is followed by the hydrolysis of the ester functional groups, in particular by the action of an alkaline carbonate or hydroxide in an alcoholic, aqueous/alcoholic or aqueous medium.

A person skilled in the art will select, during preliminary trials, the concentration of the solution, the pH, the temperature and the duration of the heating in order to carry out complete isomerization without significant decomposition, in particular according to the product and the amount treated.

It is surprising that, under these conditions, the chelate is not decomplexed and that the decomposition of the ligand is negligible and that, in addition, the pair of enantiomers which is finally isolated comprise less than 15% of the 3 pairs formed on conclusion of a conventional synthesis, which consists in hydrolysing, in basic medium, the product obtained by reaction of ethyl 2-bromoglutarate with the heterocycle and in then carrying out its complexation by the action of $GdCl_3$ or Gd_2O_3 .

Thus, according to another of its aspects, the present invention relates to a process comprising the stages consisting:

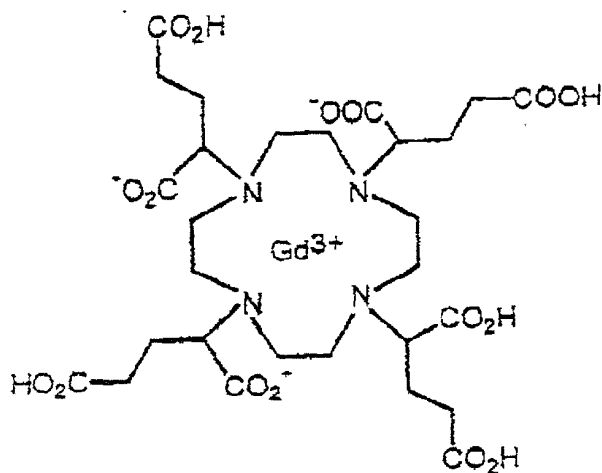
1 - in keeping an aqueous solution of the mixture of the stereoisomers of the gadolinium complex of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(2-glutaric acid), with a pH of between 2 and 4.5, at a temperature of greater than $70^\circ C$ for a few hours to a few days, so as to obtain the racemic mixture of octaacids of formula:



2 - in reacting this mixture with the amine RNH_2 , R being defined above for the formula III, with an agent which activates the acid functional group.

5

The starting mixture of the stereoisomers of the gadolinium complex of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(2-glutaric acid) of formula:



10 can be obtained in a simple fashion by employing a process comprising the stages consisting in:

- reacting 1,4,7,10-tetraazacyclododecane with a compound of formula $R'OOC-CHX(CH_2)_2-COOR'$ in which R' is a hydrogen atom or (C_1-C_3) alkyl and X is a leaving group;

15

- conventionally hydrolysing the ester functional group of the resulting compound when R' is other than H; and
- complexing the compound thus obtained with the gadolinium ion.

20

Mention may be made, as leaving group which can be used, of the sulphonate, tosylate and triflate groups.

25

The invention also relates to compositions for nuclear magnetic resonance medical imaging which comprise the racemic compounds of the invention in combination with conventional vehicles and additives. The doses at which these contrast agents will be administered depend on

their magnetic efficiency, on their biodistribution and on their administration route, as on the size of the subject, on the organ to be observed and on the nature of the pathology. For an intravascular administration, the unit concentration will be between 0.5 and 5 mM for an adult, presented in aqueous solution.

In that which follows, examples of the preparation of the compounds of the invention are described.

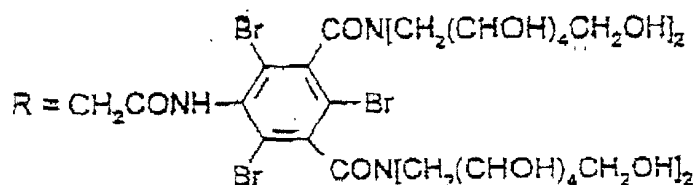
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The isolated products are characterized by their retention times (t_r) in high performance liquid chromatography (HPLC). Their molecular masses were determined by mass spectrometry (electrospray).

15

Example 1

Compound of formula II in which



20 A. Gadolinium chelate of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(2-glutaric acid) (mixture of the 6 diastereoisomers).

1. - 30 g of sodium carbonate and then 78 g of ethyl 2-bromoglutarate, prepared, for example, as described in Acta Chim. Acad. Sci. Hung., 41(3), 331-6 (1964), are introduced into a solution of 25 g of 1,4,7,10-tetraazacyclododecane in 280 ml of acetonitrile. The medium is brought to its reflux temperature for one day, during which 78 g of the brominated derivative with 30 g of sodium carbonate are added on two occasions. After cooling, the precipitate is filtered off and the organic phase is washed with water before being extracted with a dilute aqueous hydrochloric acid solution. The aqueous phase, brought to approximately

pH 3-4, is subsequently extracted with toluene.

The desired product is purified by silica chromatography, elution being carried out with
5 methylene chloride, optionally as a mixture with acetone.

2. - Hydrolysis of the ester functional groups:

10 46 g of the octaester are introduced into a solution of 52 ml of ethanol and 350 ml of water, to which 50 g of NaOH pearls have been added.

After stirring for two days at 80°C, 500 ml of cation
15 exchange resin in the weak acid form are introduced into the cooled solution for neutralization and then, after separation of the solid phase, 500 ml of anionic exchange resin in the strong base form are introduced. The resin is separated and introduced into 500 ml of 6N
20 aqueous acetic acid solution; the final product, which has passed into solution, is isolated in the form of a powder by evaporating the solvent under vacuum.

HPLC: 25 cm x 4.6 mm column of Nucleosil® C18 - 100-5 µm silica gel.

25 Eluent: aqueous H₂SO₄ (0.1%) for 10 minutes and then with 0 to 10% (v/v) of CH₃CN over 10 minutes: flow rate = 1 ml/min; T = 25°C;

t_r = 5.4, 8.7, 10.2 and 14 minutes (isomers)

(CH₃COOH - t_r = 4.5 minutes).

30

3. - Complexation:

With gadolinium oxide: 0.47 g of gadolinium oxide is introduced into 30 ml of a solution, at a pH of 5.5 to
35 6, of 2 g of the preceding octaacid and the mixture is maintained at 80°C for 3 hours, during which the pH is adjusted, if necessary. The medium is filtered, concentrated to a third and then poured into 100 ml of ethanol. The precipitate formed can be purified by

treatment with a weak basic resin before another precipitation from ethanol.

5 With gadolinium chloride: the mixture of 6.5 g of the octaacid and 3.5 g of $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ in 130 ml of water is brought to pH 6.5 by addition of aqueous NaOH (1N) and is brought to 60°C for 2 hours, during which the pH is maintained at 6.5 by addition of a total of 21 ml of 1N aqueous NaOH. After a few hours at ambient temperature, 10 the mixture is concentrated to 25 ml and the final product is precipitated from 250 ml of $\text{C}_2\text{H}_5\text{OH}$ before being purified.

HPLC: 25 cm x 4 mm Symmetry® - RP 18 - 5 μm column (Waters®)

15 UV detector at 200 nm

mobile phase: 0.037N aqueous H_2SO_4 with CH_3CN gradient (from 0% to 20% over 60 minutes); flow rate 1 ml/minute

pair of isomers (a) (30%)* t_r = 28-32 minutes

pair of isomers (b) (65%)* t_r = 32-36 minutes

20 pair of isomers (c) (5%)* t_r = 37-41 minutes

* percentage in the mixture, expressed with respect to the areas under the curve.

B. Isomerization of the preceding mixture:

25

A solution of 10 g of the preceding mixture in 100 ml of refluxing water is acidified by addition of HCl (1N) to pH 3. After stirring for 42 hours at this temperature, the solution is concentrated under reduced 30 pressure to a volume of 10 ml and left to return to ambient temperature. 6 g of precipitated final product are isolated by filtration, which product comprises a trace of isomers (b). It can be purified by recrystallization from water.

35

If heating is carried out at only 80°C, 30% of the pair (b) still remains after heating for 150 hours and 10% still remains after 400 hours.

C. Amidation:

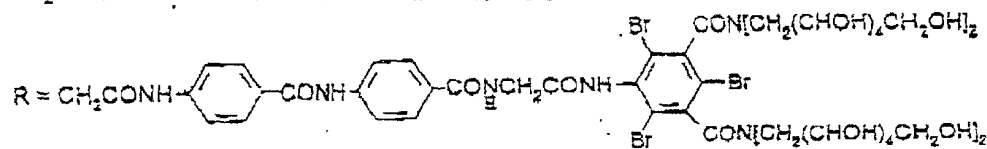
- 0.46 g of the pair of isomers obtained above and 2 g of N,N'-bis(2,3,4,5,6-pentahydroxyhexyl)-2,4,6-tribromo-5-(glycylamino)isophthalamide (compound IID of WO 97/01359) are dissolved in 8 ml of water, and a 6N aqueous NaOH solution is poured into the medium to pH 6 before introducing, at 40°C, 0.48 g of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.
- The medium is maintained at 40°C for 2 hours with stirring while introducing, from time to time, an N aqueous NaCl solution in order for the pH not to exceed 7.
- After returning to ambient temperature, the solution is poured into 100 ml of ethanol and the precipitate formed is isolated and then dissolved in 100 ml of water to produce a solution. This solution is subjected to tangential ultrafiltration through a polyether-sulphone membrane, the cutoff threshold of which is 1 Kdalton, in a Minisette® cell sold by Filtron®, USA.

After lyophilization, 1.5 g of the final product are isolated in the form of a white powder.

- HPLC: 25 cm x 4 mm Symmetry® - RP 18 - 5 µm column (Waters®)
- UV detector at 230 nm
- mobile phase: 0.037N aqueous H₂SO₄ with CH₃CN, gradient from 99/1 to 90/10 (v/v) over 25 minutes, flow rate 1 ml/minute,
- $t_r = 16$ to 20 minutes (several peaks).

Example 2

- Compound of formula II in which



A. N,N'-bis(2,3,4,5,6-pentahydroxyhexyl)-2,4,6-tri-bromo-5-(4-[4-(aminoacetamido)benzamido]benzoyl)glycyl-amino)isophthalamide.

5 (a) 4-[4-Nitrobenzamido]benzoic acid:

100 g of 4-nitrobenzoyl chloride are introduced, little by little, into 74 g of 4-aminobenzoic acid and 360 ml of dimethylacetamide while maintaining the temperature at less than 25°C. After stirring for 24 hours, 500 ml of methylene chloride are added at 10°C to precipitate the desired product. After washing with water and drying, 145 g of product are isolated.

15 (b) 4-[4-Aminobenzamido]benzoic acid;

A suspension of 136 g of the preceding acid in 1.8 litres of water, to which 240 ml of 1N aqueous NaOH solution and 14 g of palladium-on-charcoal (10%) have been added, is subjected to a hydrogen pressure of 0.6 MPa for 4 hours. The pH of the final suspension is then brought to approximately 10 before filtering through Celite® to remove the catalyst. The precipitate formed during the acidification of the filtrate to pH 5.3 is isolated and dried.
w = 106 g; M.p. > 260°C.

(c) 4-[4-(Phthalimidoacetamido)benzamido]benzoic acid:

30 32 ml of thionyl chloride are introduced dropwise into a solution of 90 g of phthalimidoacetic acid in 400 ml of dimethylacetamide at 10°C and then, after stirring for 3 hours, 105 g of the amino acid obtained above are introduced at a temperature of less than 20°C. After stirring for 12 hours, the medium is poured into 4 litres of water and the isolated precipitate is washed with hot water.
Weight after drying: 176 g; M.p. > 260°C.

(d) Chloride of the preceding acid:

2.5 ml of thionyl chloride are introduced into 10 g of the acid, in suspension in 50 ml of dioxane and 1 ml of dimethylformamide, and the mixture is kept stirred at 50°C for 5 hours. After addition of one volume of diisopropyl ether, 10 g of precipitate are isolated.

The acid can also be suspended in toluene with tricaprylmethylammonium chloride as catalyst.

(e) N,N'-Bis(2,3,4,5,6-pentahydroxyhexyl)-2,4,6-tribromo-5-(4-[4-(phthalimidoacetamido)benzamido]-benzoylglycylamino)isophthalamide:

A solution of 2.25 g of acid chloride with 5 g of N,N'-bis(2,3,4,5,6-pentahydroxyhexyl)-2,4,6-tribromo-5-(glycylamino)isophthalamide and 0.7 ml of triethylamine in 25 ml of N-methylpyrrolidone is kept stirred for 12 hours; the $(C_2H_5)_3N \cdot HCl$ precipitate is then separated by filtration.

(f) Hydrazinolysis:

A solution of 1.4 equivalents of hydrazine hydrate in 6 ml of water is introduced into the preceding phthalimide solution at 70°C. After stirring for 2 hours at 90°C, the cooled mixture is poured into 125 ml of ethanol. 9 g of precipitate are isolated, from which the phthalylhydrazide is separated by precipitation of an aqueous solution at pH 2, before ultrafiltration at pH 6 through a polyamide membrane to remove the impurities of low mass. The final hydrochloride is subsequently isolated by lyophilization.

Yield: 50% from the acid chloride.

HPLC: 25 cm x 4 mm Lichrospher® 100-Å - C18 - 5 µm column (Merck, Germany).

Eluent: CH_3COOH in H_2O (pH 3.3) and CH_3CN (90/10 v/v); flow rate 1 ml/min;

t_r = 22, 24 and 27 minutes (3 peaks).

5 B. 0.28 g of the complex obtained in stage (B) of the preceding example and 2 g of the hydrochloride obtained in the preceding stage (A) are dissolved in 12.4 ml of water and the pH of the solution is brought to 6 by addition of N aqueous NaOH before adding 10 ml of a solution in dioxane of 0.2 g of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.024 g of hydroxybenzotriazole.

15 The solution is then stirred for 4 hours at ambient temperature while maintaining its pH at approximately 6, before being poured into 100 ml of ethanol. The precipitate formed is dissolved in 100 ml of water and the solution is ultrafiltered through a polyethersulphone membrane with a cutoff threshold of 30 Kdaltons.

20 After removing the solvent, 1.3 g of the desired product are obtained in the form of a white powder.

HPLC: 25 cm x 6 mm Zorbax® - 300 5B - C18 - 5 μ m column (Hewlett-Packard)

UV detector: 290 nm

25 Eluent: aqueous $\text{CH}_3\text{COONH}_4$ (0.005M) with a CH_3CN gradient (90/10 to 82/18) (v/v) over 60 minutes; flow rate 1 ml/min;

t_r = 30 to 40 minutes (several peaks).

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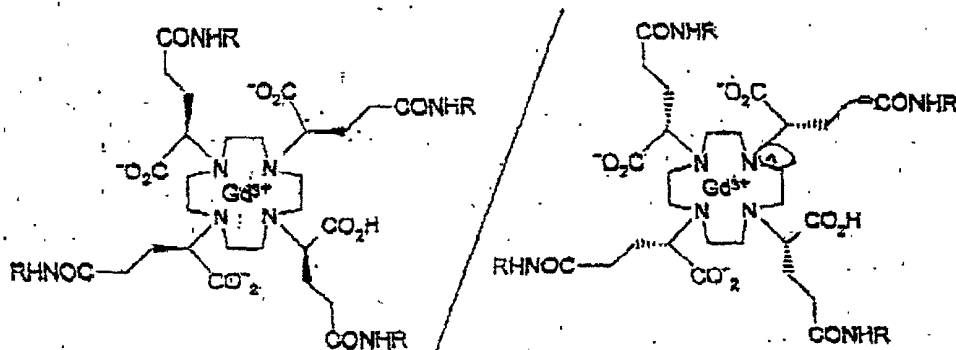
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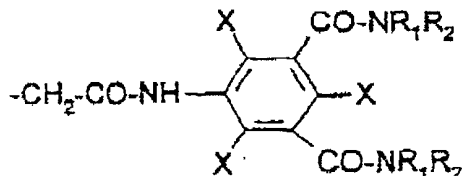
Claims

1. Contrast agent for medical imaging, characterized in that it comprises a racemic compound of formula A



- in which R is a phenyl group or (C₁-C₈) alkyl group which are substituted or interrupted by one or more groups selected from phenyl, alkyl, oxy, amino or amido groups, which may or may not be substituted by alkyl,
it being possible for the phenyl groups also to be substituted by OH, Br, Cl, I, (C₁-C₈)alkyl, (C₁-C₈)alkyleneoxy, NO₂, NR_xR_y, NR_xCOR_y, CONR_xR_y or COOR_x, R_x and R_y being (C₁-C₈)alkyl or H,
and it being possible for the linear or branched or cyclic alkyl groups to be hydroxylated,
and the salts of this acid with a physiologically acceptable inorganic or organic base.

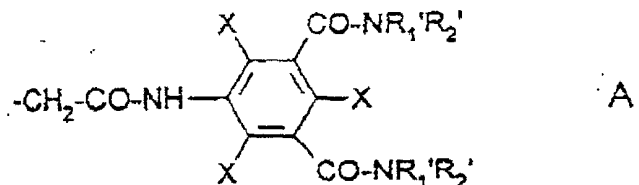
2. Contrast agent according to Claim 1, for which R is a group of formula



in which

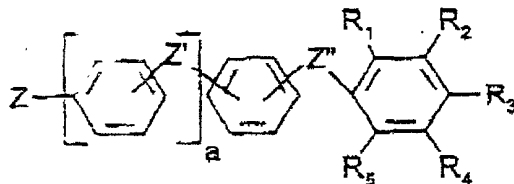
- X is Br or I, R₁ is H, (C₁-C₃)alkyl or (C₂-C₈)mono- or polyhydroxyalkyl and R₂ is (C₂-C₈)mono- or polyhydroxyalkyl, or else R₁ is H and R₂ is a group

of formula



X being as defined above and R₁ and R₂ taking any one of the meanings given for R₁ and R₂, with the exception of A, it being understood that -CO-NR₁R₂ or -CO-NR₁R₂' comprise at least two hydroxyl groups, and its salts with a physiologically acceptable inorganic or organic base.

3. Contrast agent according to Claim 1, for which R is a group of formula



in which a is 1 or 2,

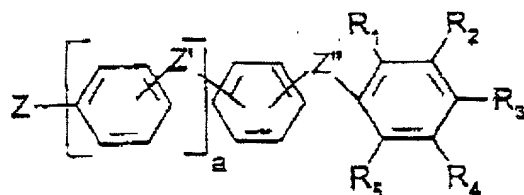
Z is a bond, CH₂, CH₂CONH or (CH₂)₂NHCO,

Z' is a bond, O, S, NQ, CH₂, CO, CO-NQ, NQ-CO, NQ-CO-NQ or CO-NQ-CH₂-CONQ,

Z'' is CO-NQ, NQ-CO, CO-NQ-CH₂-CO-NQ or NQ-CO-CH₂-NQ-CO,

with Q being H or an optionally hydroxylated (C₁-C₄)alkyl group,

R₁, R₂, R₃, R₄ and R₅, independently of one another, are selected from H, Br, Cl, I, CO-NQ₁Q₂ or N(Q₁)-CO-Q₂, and Q₁ and Q₂, which are identical or different, are selected from optionally hydroxylated (C₂-C₆)alkyl groups optionally interrupted by an oxygen atom, so that Q₁ and Q₂ together comprise from 4 to 10 OH groups, it being understood that at least 1 and at most 2 R₁, R₂, R₃, R₄ and R₅ groups are amide groups.



in which a is 1 or 2,

Z is a bond, CH_2 , CH_2CONH or $(\text{CH}_2)_2\text{NHCO}$,

Z' is a bond, O, S, NQ, CH_2 , CO, CO-NQ, NQ-CO, ...

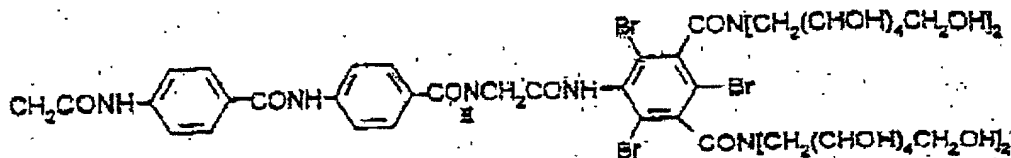
5 NQ-CO-NQ or CO-NQ- CH_2 -CONQ,

Z'' is CO-NQ, NQ-CO, CO-NQ- CH_2 -CO-NQ or
NQ-CO- CH_2 -NQ-CO,

with Q being H or an optionally hydroxylated
(C_1 - C_4)alkyl group,

10 R_1 , R_2 , R_3 , R_4 and R_5 , independently of one another,
are selected from H, Br, Cl, I, CO-NQ $_1$ Q $_2$ or N(Q $_1$)-
CO-Q $_2$, and Q $_1$ and Q $_2$, which are identical or
different, are selected from optionally
15 hydroxylated (C_2 - C_6)alkyl groups optionally
interrupted by an oxygen atom, so that Q $_1$ and Q $_2$
together comprise from 4 to 10 OH groups,
it being understood that at least 1 and at most 2
 R_1 , R_2 , R_3 , R_4 and R_5 groups are amide groups.

20 8. Racemic compound according to Claim 7, in which R
is a group of formula



DECLARATION
AND POWER OF ATTORNEY
U.S.A.

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ATTORNEYS' DOCKET NO.

ALL PATENTS, INCLUDING DESIGN
FOR APPLICATION BASED ON PCT; PARIS CONVENTION;
NON-PRIORITY; OR PROVISIONAL APPLICATIONS

As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201 below), or an original, first and joint inventor (if plural inventors are named below at 201-203, or on additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled:

"Tetramides of a gadolinium complex and application in medical imaging".

which is described and claimed in:

☒ PCT International Application No. PCT/FR00/01382

filed on May 19, 2000

☒ the attached specification

☐ the specification in application Serial No. _____

filed _____

(if applicable) and amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

99 06517

FRANCE

21/05/99

(Number)

(Country)

(Day/Month/Year Filed)

Priority Claimed

☒ Yes

☐ No

(Number)

(Country)

(Day/Month/Year Filed)

☐ Yes

☐ No

(Number)

(Country)

(Day/Month/Year Filed)

☐ Yes

☐ No

I hereby claim the benefit under Title 35, United States Code, §119(s) of any United States provisional application(s) listed below:

Application No. _____

Filing Date _____

Application No. _____

Filing Date _____

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)

(Filing Date)

(Status: patented, pending, abandoned)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys (Registration No.) to prosecute this application, receive and act on instructions from my agent, and transact all business in the Patent and Trademark Office connected therewith. HARVEY B. JACOBSON, JR. (20,851); D. DOUGLAS PRICE (24,514); JOHN CLARKE HOLMAN (22,769); MARVIN R. STERN (20,840); ALLEN S. MELSER (27,215); MICHAEL R. SLOBASKY (26,421); JONATHAN L. SCHERER (29,851); IRWIN M. AISENBERG (19,007); WILLIAM E. PLAYER (31,409); YOON S. HAM (45,307) and NATHANIEL A. HUMPHRIES (22,772)

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*Inventor(s) name must include at least one unabbreviated first or middle name.

201	FULL NAME* OF INVENTOR	FAMILY NAME <u>ROUSSEAU</u>	GIVEN NAME <u>Olivier</u>	MIDDLE NAME
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202	FULL NAME* OF INVENTOR	FAMILY NAME <u>SIMONOT</u>	GIVEN NAME <u>Christian</u>	MIDDLE NAME
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203	FULL NAME* OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201*	SIGNATURE OF INVENTOR 202*	SIGNATURE OF INVENTOR 203*
<u>Rousseau</u>	<u>Simonot</u>	
DATE <u>18-09-2001</u>	DATE <u>18 September 2001</u>	DATE

☐ Additional inventors are named on separately numbered sheets attached hereto.

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